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Study of the Acidity of Beef Extract

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A STUDY

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ACIDITY OF BEEF EXTRACT

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY
and that of Professor Bindley.

John Albert McFarland

ENTITLED A Study of the Acidity of Beef Extract

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF Bachelor of Science

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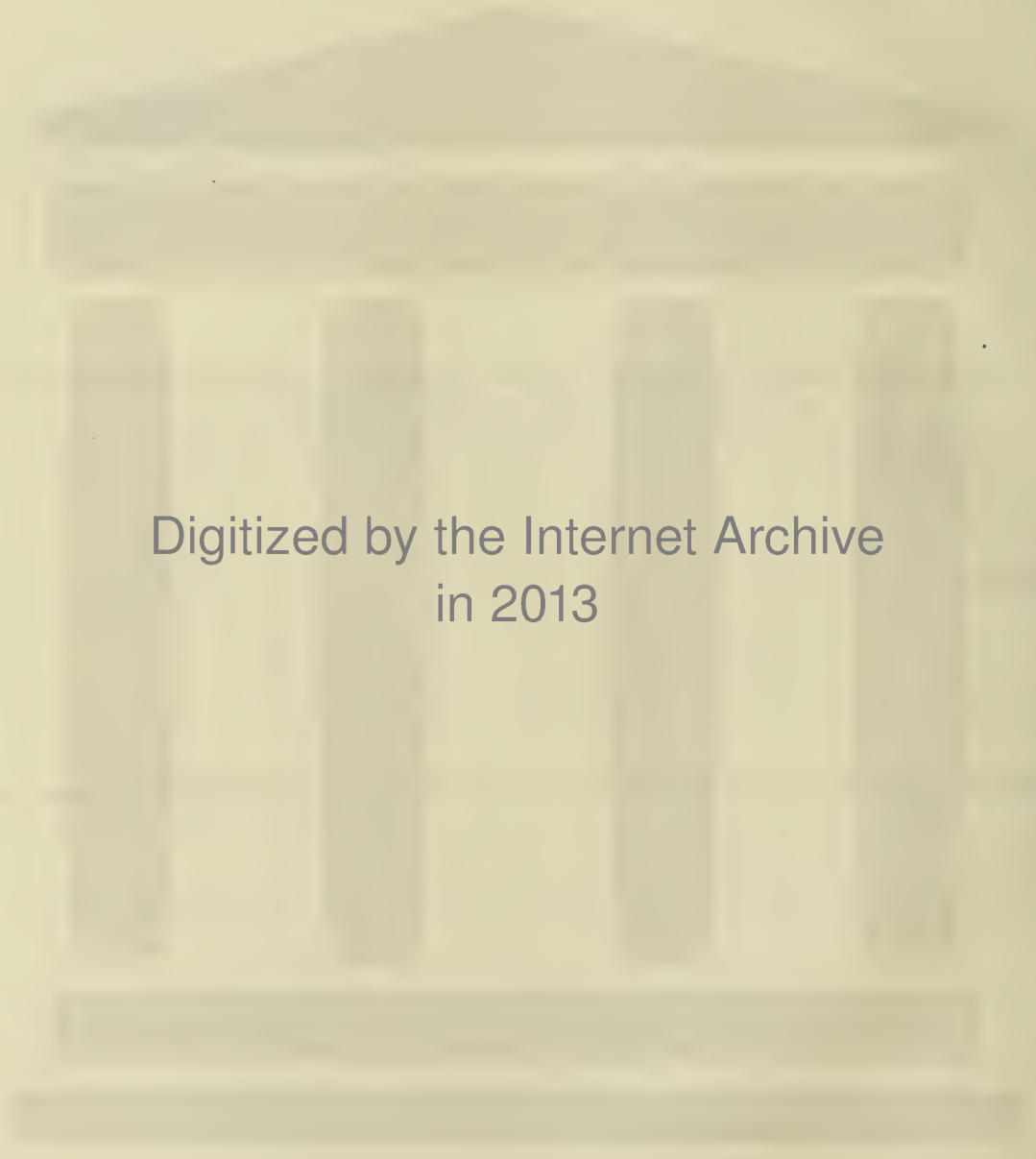

A STUDY OF THE ACIDITY OF BEEF EXTRACTS

COMPOSITION OF BEEF EXTRACT

The composition of meat extracts varies between wide limits and even the same make of extract varies in composition from time to time. There are many circumstances which are likely to affect the composition of the preparation; among these are the age of the animal; the length of time between the slaughtering and the beginning of the extraction; and the use of different cuts of flesh. Qualitatively, the composition of meat extracts is comparitively uniform, but this is not always the case. The principal constituents of meat extracts may be classed as in the table upon the following page. (See Table No. I) (1)

Extract of meat was first described by Proust in 1801, but the method of manufacturing it on a commercial scale is due to Liebig, who described the method in 1847. In all this time little investigation of value was done. In 1847 Liebig, whose name stands preeminent in the early investigation of meat extracts, published the results of his work. This date marks the beginning of anything like accurate and valuable investigations upon this subject. He attributed a relatively greater nutritive value to the nitrogenous material than is given today. In his paper (2) he gives a table which states the constituents of lean beef and their relative quantities as then determined. (See Table No. II)

Among the constituents which he separated and studied, he mentions,- creatin, creatinin, sarcosine, inosinic acid and inorganic constituents. From one hundred pounds of flesh of a lean old horse

Table No. I.

Constituents of meat extracts.

I. Water.

II. Mineral matters.

1. Phosphates of potassium, calcium, sodium.

2. Chlorides of potassium and sodium.

3. Potassium and sodium salts of organic acids.

III. Organic matter.

1. Proteids.

Meat-fibre, albumins and globulins,

syntonin, gelatin and albuminoses,

peptones.

2. Meat bases.

Creatin, creatinin, xanthin, hypoxanthine,

guanine, carnine, leucin, carnosin.

3. Free and combined organic acids, e.g.

lactic acid and lactates.

4. Glycogen and inosite.

5. Unknown constituents.

he obtained thirty-six grams of creatin; and eighty six pounds of ox flesh gave thirty grams of creatin. No figures for the other constituents were given.

Until comparatively recent years, analyses of meat extracts consisted of empirical determinations whereby a comparison between two extracts might be made. In the light of present knowledge, however, it is seen that the conclusions and interpretations which were derived from the older analyses were often erroneous.

Until within the last few years analyses of beef extract were limited to determinations of water, organic matter, ash, soluble albumin, and alcoholic extract. The following table (3) gives some analyses of extracts sold in New York State in 1882. A mere inspection of the data serves to show how crude and unsatisfactory were the analyses upon which judgements of meat extracts were at that time based. (See Table No. III) The only determinations which are of any value in these analyses are the "water" and "ash", and these are of no value in discovering the nature of the organic matter. The strength of the alcohol used to obtain the results in the last column is not stated, and the determination is without value, since it is not known what the value of the constituents extracted by alcohol really is.

It is important to determine the water since this constituent varies within wide limits.

Lehner Lehnes (4) when investigating the method of proteid precipitation by means of alcohol obtained the following percentages of water:- Liebigs extract 18.70 percent, concentrated beef tea, English, 41.97 per cent, Russian 24.56 per cent, Commercial essence

Table No. II.

The constituents of lean beef and their relative quantities as determined in the time of Liebig.

From 1000 grams of lean beef:-

I.	Soluble in cold water.		
	Coagulable, albumins	29.5 grams.	
	Non-coagulable	30.5 grams.	
	Total	60.0 grams.	
	Total soluble in cold water		60.0 grs.
II.	Insoluble in water.		
	Gelatin	6.0 grams.	
	Insoluble proteids	164.0 grams.	
	Total	170.0 grams	
	Total insoluble in water		170.0 grs.
III.	Fat		20.0 grs.
IV.	Water		750.0 grs.
	Total		1000.0 grs.

Table No. III.

Analyses of beef extracts sold in New York State in 1882.

Name of extract.	Water.	Organic matter.	Ash.	Soluble albumin.	Alcoholic extract.
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1. Liebig's extract	13.27	58.48	28.25	0.05	44.11
2. Berger's extract	40.65	39.35	19.50	1.11	17.18
3. Starr's extract	37.00	55.65	7.35	1.10	10.17
4. Johnston's fluid beef	41.20	50.40	8.40	1.17	15.93
5. Grant's beef peptone	37.15	54.92	7.93	0.00	20.16
6. Valentine's meat juice	54.40	31.85	13.75	0.44	26.52

of beef, English, 92.72 per cent, South African, 87.55 per cent.

The mineral material constituents vary of course with the concentration of the extract. Hehner's table (5) shows the following:- Brand and Co's Essence of Beef 1.0 per cent, Vetalia Meat juice, 6.05 per cent, Bovril for Invalids 16.50 per cent, Bothwick's bouillon 17.03 per cent, Liebig and Co's Extraction, Carnis 27.51 per cent, and Armour's Extract of Meat 29.36 per cent.

Allen (6) says that the salts of meat extract consist chiefly of earthy phosphates, potassium chloride and acid phosphates. There are also present lactates and other organic salts of potassium. He gives the mineral constituents of fresh meat based upon 100 parts of meat. This is given in table No. IV.

From a thorough study of the literature upon the subject it is very evident that even the most detailed analyses made of meat extracts hitherto are only approximately accurate.

Table No. IV.

Mineral constituents of fresh meat based on 100 parts of meat.

I. Soluble.			
	Potassium chloride	.112	
	Sodium chloride	.023	
	Potassium sulphate	.055	
	Secondary potassium phosphate	.409	
	Neutral calcium phosphate	.018	
	Neutral magnesium phosphate	.036	
	Total	.653	
	Total soluble		.653
II. Insoluble.			
	Calcium and magnesium phosphates	.440	
	Total insoluble		.440
	Total		1.093

UNKNOWN CONSTITUENTS OF BEEF EXTRACT.

The non-nitrogenous organic matter including glucose, milk, sugar, fat, inosite, and glycogen have not been given much attention in the past but of late they are receiving greater consideration. In most cases where these constituents have been estimated they appear under the head of non-nitrogenous extractive matters and are obtained in many cases by difference. That is the sum of the percentages of the constituents determined does not equal one hundred, and as the methods for the determination of the quantities of organic matter are not well developed the difference between one hundred per cent and the sum obtained by actual analysis is often attributed to these extractive matters. This point is illustrated by Table No. V. (7)

Gautier's results which are the best which we have up to the present time are only averages of the composition of the extract of a litre of broth. Even it shows extractive matter equalling more than fourteen per cent. The term "extractive matter" serves to cover the constituents determined by difference.

A. Gautier's work (8) showed the nitrogen to be distributed in meat broth as follows:-

Peptones and propeptones,	28.8% of solids.
Gelatinoids,	14.1% of solids.
Creatin and other bases	10.9% of solids.

He found eight per cent of the solids obtained from broth to consist of inosite, glycogen, and other reducing material, about fourteen per cent extractives, and twenty-four per cent salts, the remainder (fifty-three and eight tenths per cent), being

Table No. V.

Table showing the extractive matters.

Constituents.	Liebigs and Co's extract.	Bovil fluid beef seasoned.	Bovril for invalids.	Brand's and Co's beef bouillon.	Esco beef juice.	Vimbos.	Viking beef essence.
Water	18.35	75.10	21.16	56.27	52.43	74.95	90.68
Insoluble proteids meat fibre etc.	Trace	3.40	3.47	3.48	0.56	7.23	None
Soluble proteids and gelatins	9.45	3.84	8.19	5.73	7.10	4.23	3.63
Flesh bases (N x 3.12)	29.67	12.04	16.13	19.34	5.90	12.60	1.35
Non-nitrogenous extractive matters	18.35	19.75	29.23	19.75	13.62	27.61	2.41
Mineral	23.68	17.87	16.82	15.07	20.79	17.35	1.43
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

nitrogenous matter distributed as given in the proceeding table.

Porter (9) attempted to separate and determine the different nitrogenous compounds. He found by a summation of the constituents, that is by a summation of salts, ether extract, and nitrogenous matter, that he had accounted for about seventy-five per cent of the total solids in the clear broth.

Table VI, which gives the results obtained in this laboratory by Porter, Emmett and Grindley, shows that there is practically the same amount of undetermined constituents in meat broth. The percentage of undetermined constituents however, varies from twenty to forty per cent in the different samples.

Emmett (10) began the study of meat broths with the purpose if possible of isolating and estimating the material otherwise estimated by difference. Some of the problems he investigated are:- The proper factor for calculating the flesh bases. The possibility of error due to the methods of analysis, and the possibility of water being held mechanically by the total solids. He concluded that neither of the above reasons was sufficient to account for the difference and states in his resumé that it is due at least in part to the non-crystallizable organic matter of alkaloidal properties containing a small percentage of nitrogen.

Williams (11) in the course of his work found that it was very difficult to separate and identify the organic nitrogenous matters and could find no good method for the separation. He found the quantity of combined lactic acid or lactates to be larger than has heretofore been supposed. This will in a measure account for the undetermined matter.

Table No. VI.

Constituents of beef broth.

Lab. No.	Total solids by direct determination.	Protein. (N x 6.25)	Flesh bases. (N x 7.12)	Fat.	Ash.	Total by summation.	Other substances by difference.	Per cent difference.
	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	
777a	20.388	2.628	5.58	2.776	5.016	15.988	4.400	21.59
779a	20.140	2.964	5.480	2.800	4.832	16.076	4.064	20.16
781a	17.216	2.176	4.416	0.532	4.010	10.934	6.282	37.11
809a	18.416	1.072	5.376	0.392	4.576	11.416	7.000	38.02
892	21.230	1.700	6.501	1.924	5.464	15.189	6.041	28.46
893	25.756	2.476	7.775	21.160	6.140	18.111	7.245	28.65
894	12.668	0.542	3.869	1.016	3.170	8.597	4.071	32.14
895	51.128	2.478	15.305	3.938	2.276	34.042	17.086	33.42
1146	15.083	0.875	4.910	0.143	3.623	9.551	6.432	71.57
1147	41.907	2.084	12.745	0.091	10.170	25.090	16.817	38.87
1158	22.085	1.059	6.408	0.232	4.313	12.517	9.568	44.32
1160	14.014	1.144	3.864	0.214	2.996	8.218	5.796	41.36

ACIDITY OF BEEF EXTRACT.

The flesh of dead animals however fresh flesh in the ordinary sense has an acid reaction; Berzilius (12) who discovered this fact concluded that the acidity was due to lactic acid. This lactic acid was shown by subsequent researchers to differ from the lactic acid produced by fermentation. Liebig first denied but afterwards affirmed its presence in flesh. All the early chemists thought that lactic acid was normally present in the tissues during life, since they always found it in the flesh of recently killed animals. At that time the conception had not been formed that when death sets in certain processes start that give rise to the formation of new substances which are products of decompositions of the flesh. This conception was due to Du Bois - Reymond (13). He showed the importance of distinguishing between a tissue which is yet living, thought it may be separated from the living body of which it once formed a part, and one which has ceased to manifest the phenomena which it manifested during life. There is a change in chemical and physical properties as soon as these phonemna cease,- in warm blooded animals almost instantly. The belief now is that when a muscle is alive it possesses a neutral reaction; when it dies it becomes acid. In warm blooded animals the change goes on so quickly that it is almost impossible to determine the normal reaction. In cold blooded animals the acidification goes on slowly enought to permit of its careful study.

Nasse (14) believed that the acidity was due to the decomposition of glycogen in the muscle. Since then almost all observers have come to the belief that proteids are its source.

Böhn (15) found the same amount of glycogen in rigovized and putrified muscle as in the flesh, and hence concluded that it could not be the source of the acid. The formation of acidity almost simultaneously with coagulation, would tend to show, that the acidity comes from the proteids. In addition to the lactic acid and perhaps some of the other organic acids, some of the acidity is due to the acid phosphates formed from the neutral or alkaline phosphates by the development of new phosphoric anhydride from lecithin.

Mojonnier (16) made a cold water extract of fresh beef and found the extract to be distinctly acid to phenolphthalein. On acount of the concentration of the extract he diluted a measured quantity with water before making the titration. He titrated with a solution of standard sodium hydroxide and found that on boiling and thus upon coagulating the albumin that the acidity of the solution kept increasing step by step, and finally ceased when the albumens were completely precipitated. When no further precipitation of albumin resulted then the solution remained neutral. Mojonnier titrated a cold water extract of beef with standard sodium hydroxide containing .00539 grams of sodium hydroxide equivalent to .01214 grams of lactic acid per cubic centimeter. Five hundred cubic centimeters required fifteen cubic centimeters of the standard sodium hydroxide. The solution was evaporated to a small volume and filtered. A little more phenolphthalein was added to this filtrate and the solution again titrated. It required four and one tenth cubic centimeters of the standard sodium hydroxide to neutralize it. The solution was then heated to boiling, filtered, the precipitate

washed with hot water, and the filtrate and washings titrated again. Two and one-tenth cubic centimeters of standard sodium hydroxide were used this time. The neutralized filtrate was evaporated to small volume. This time no precipitate formed and the solution remained neutral. Thus it was shown that the acidity of the solution was increased about one-half by the boiling and coagulation of the albumins. Hojonnier found the acidity of a water extract of fresh beef to vary from seventy-one hundredths of one percent to one and thirty hundreths percent calculated to lactic acid.

From recent researches T. Irisawa (17) concludes that lactic acid is always present in blood removed from the dead body, and in three cases out of seven it was present shortly before death. Lactic acid was always present in blood freshly drawn from the veins of a dog, and its presence was also noted in pus and in blood corpuscles.

Nebelthau (18) obtained 0.127 grams of zinc lactate from seven and eight tenths litres of urin obtained from 265 frogs in four days.

Zillessen (19) found lactic acid in the urin of dogs and rabbits especially when the action of the liver was interfered with.

Minkowski (20) observed that the separation of lactic acid in the urin entirely disappeared when the livers of duck were destroyed.

Salenskin and Zaleski (21) kept a dog alive thirteen hours after the separation of the liver and observed the changes in the chemical composition of the urin. They came to the conclusion that the formation of lactic acid is in some way connected with the disturbance of the activities of the liver.

Araki (22) proved positively that with a difficiency of oxygen in the blood, no matter how it is occasioned, lactic acid appears in the urin of rabbits in notable quantities.

Münzer and Palma (23) proved in the case of people poisoned by carbon monoxide that this is actually the case.

Araki
Arki (i.e. 22) found that in hungry animals, lactic acid was contained in the urin but in well fed animals lactic acid and sugar was found in the urin. This, however, has not been absolutely proven as Araki did not analyze the blood of the chicken or analyze the zinc salt.

Saito and R. Katsuyama (24) say that the lactic acid must first be formed in the blood and that this is the direct cause of its appearance in the urin.

Berlinerblau (25) succeeded in proving with absolute certainty the presence of sarco lactic acid in normal rabbit and dog blood. He found it even in human blood. In two-hundred cubic centimeters of venous human blood which he caught directly in alcohol, he found, .1562 grams of lactic acid.

Gaglio (26) also found lactic acid .39 of one percent in the blood of a chicken fed upon meat.

S. Saito and R. Katsuyama found that normal hens blood contains about thirty nine hundredths of one percent of lactic acid.

The method which S. Saito and R. Katsuyama used for the determination of lactic acid is the same as is used by other investigators and is as follows:- Blood which was taken from the neck or heart of the animal was weighed, then diluted with six times its the volume of ninety six per cent alcohol, and after standing twelve hours, with frequent stirring was filtered. The residue was washed

four or five times with ninety six per cent alcohol and the residue pressed out with a filter press and discorded. The alcohol was distilled from the alcoholic extract. The residue from the alcoholic extract was dissolved in a little water, the solution was made alkaline with several drops of a solution of soda. In order to separate the fat the liquid was shaken up five times, with new volumes of ether. The fat free solution was now made acid with the same volume of moderately dilute phosphoric acid and shaken up six times with five times its volume of ether. The syrup which remains after the ether has been distilled off was neutralized with barium hydroxide, filtered and washed. The excess of barium hydroxide in the filtrate was removed by the addition of dilute sulphuric acid and the solution then concentrated upon the water bath, and then extracted with ether. From the clear decanted ether extracts, the ether is distilled and the residue boiled with water and an excess of zinc oxide, the liquid filtered hot and washed well. The filtered solution is put in a weighed flask and evaporated to a small volume on the water bath, and after the addition of a few drops of alcohol, was allowed to stand for crystallization.

By this method S. Saito and R. Katsuyama found that four hundred and fifty grams of normal hens blood, from five animals yielded 0.1851 grams of zinc lactate. The zinc salt was recrystallized and showed the characteristic forms of zinc lactate. The zinc lactate prepared in this way from hens blood was identical with para lactic acid zinc salt.

The analysis of the zinc salt of the lactic acid solution from the blood of chickens poisoned with carbon monoxide give the results which will be found in table VII.

Experiment shows that the per cent of lactic acid in chicken blood is 0.0269 and the per cent after carbon monoxide poisoning is 0.1227. Thus we can see from these results that the acid is lactic acid. On the grounds of these different truths we have the right to make the following assertion. The difficulty in oxygen and total separation of the liver are two different causes which produce abnormal amounts of lactic acid in animal bodies.

It was a very difficult matter to estimate lactic acid quantitatively for as Liebig says, lactic acid does not form a single completely insoluble salt with any of the known metals. The method for the determination of lactic acid used in most cases consists in converting the acid into the zinc salt and extracting with alcohol. The lactate of the inactive acid remains insoluble. The para lactate however is soluble in alcohol and hence this method will not serve our purpose.

Very few attempts have been made to determine the acidity of beef extracts and it is undoubtedly true that the acidity of these preparations has never been accurately determined.

The acidity of beef extract is commonly considered to be due to lactic acid but as far as I could find out no one has proven that the acidity is due to lactic acid entirely and there is at present no good method for the quantitative determination of lactic acid. There are also acid phosphates present.

Urea CH_4N_2O and uric acid ($C_5H_4N_4O_3$) have been thought by some authors to exist in small quantities in muscular tissues but their presence is doubtful. Higgins (27) in his work tried to determine the amount of phosphorus in the organic compounds and

Table No. VII.

Analysis of the zinc salt of lactic acid obtained from the blood of chicken poisoned with carbon monoxide.

$(C_3H_5O_3)_2Zn + 2H_2O$

Theoretical	H_2O	12.9%
	Zn	26.70%
Found	H_2O	12.52%
	Zn	26.70%
Before use of CO	H_2O	12.98%
	Zn	26.55%

the state of combustion of the phosphoric acid. The process which he used was based on the following facts:-

First. A solution of meat extract was made ammoniacal with the production of a flocculant precipitate which he proved to be tertiary. Calcium phosphate. $Ca_3P_2O_8$.

Second. To the filtrate from the above a slight excess of an ammoniacal solution of calcium chloride was added and the solution filtered from the calcium phosphate precipitated. The filtrate was divided into two parts, (A) and (B).

In part (A) the phosphoric acid was determined by means of ammonium molybdate and "Magnesia mixture" in the usual way.

Part (B) was evaporated and ignited in a platinum dish, heated with nitric acid, and tested for phosphates with ammonium molybdate. A considerable precipitate was produced.

Higgins drew the following conclusions from the results of these tests. 1.- Neutral calcium phosphate is precipitated from a solution of meat extract by rendering alkaline with ammonia. 2.- All phosphoric acid in the form of ortho-phosphates is precipitated from the solution by ammoniacal calcium chloride. 3.- Organic phosphorus is present in considerable quantity in beef extract.

Higgins tried to determine the relative amounts of secondary and primary phosphate in Armour's Beef Extract and his results indicated that there was six times as much secondary as primary potassium phosphate.

This was the only attempt which I could find that had been made to separate the potassium salts as such. The lactic acid has been extracted by ether and then either titrated with standard

alkali or obtained in the form of the zinc lactate.

The detailed analyses of meat extracts and broths show that there is a large part, called "extractive matter", which is determined by difference. When it is noted that Williams obtained six per cent lactic acid and Higgins obtained nearly as much it can be readily seen that a part at least of the extractive matter can be accounted for by the presence of lactic acid both as free lactic acid and as salts.

One question which has been raised is as to the amount of volatile acids in meat extracts, both as free and combined volatile acids. No determinations were found upon the subject in any of the literature which I looked over so I will try to solve this question in the course of my work. I looked over the following literature:-

Higgins B.S. Thesis, 1902.

William's B.S. Thesis, 1902.

Porter's B.S. and M.S. Thesis, 1899 and 1900.

Mojonnier's B.S. and M.S. Thesis, 1901 and 1902.

Chemical News, Vol. 1 - 1860 to Vol. 84 - 1901.

Liebig and Kopp, Jahresbericht, 1847 - 1888.

Journal of the Chemical Society, London, 1849 - 1901.

Journal für Praktische Chemie, 1834 - 1902.

Berzelius Jahres - Bericht der Chemie, Vol. 1 - Vol. 30.

Monatshefte für Chemie, 1880 - 1897.

Zeitschrift für Analytische Chemie, 1862 - 1901.

Zeitschrift für anorganische Chemie, 1892 - 1901.

Bericht der Chemichen Gesellschaft, 1868 - 1896.

Analyst, 1877 - 1901.

Annalen der Chemie, Vol. 1 - 297 - 1896.

Annalen der Chemie and Pharmacie, 1894 - 1901.

Zeitschrift für Physiologische Chemie, 1877 - 1901.

Methods for determining the acidity of meat extracts.

The next question which presents itself is as to whether a method could be found by which the free organic acids and the acid phosphates could be determined. No results of the determination of the free acids in meat extract could be found in the literature at hand.

Higgins attempted to determine the free acid by titration with standard alkali. A small sample of Armour's Extract was dissolved in water, diluted until practically colorless, and titrated with deci-normal sodium hydroxide, using phenol-phthalein for an indicator, the titration calculated to lactic acid in the sample, amounted to sixteen and five tenths per cent. When he used litmus as an indicator only about half this amount was obtained. From such results of course no conclusions could be drawn. He took another sample and filtered it through animal charcoal and then titrated with deci-normal alkali using phenol-phthalein as indicator. He obtained twenty five per cent acid. He tried another determination using barium carbonate paste. He digested a sample of the beef extract with the barium carbonate paste, filtered, washed and decolorized the filtrate with animal charcoal, and then titrated with deci-normal alkali. He obtained eleven per cent of free acid calculated as lactic acid. Thus fourteen per cent acid was neutralized with barium carbonate. He also tried to see if the free lactic acid could not be determined by estimating the amount

of barium carbonate dissolved. To do this he digested a sample of the beef extract with barium carbonate paste on the water bath, for several hours, the solution was filtered, the residue washed and the barium determined in the filtrate. The per cent of barium gave three and fifteen hundredths per cent of lactic acid figured on the basis of normal barium lactate. If it was the acid barium lactate which was formed, the per cent of lactic acid would be double or six and three tenths per cent. He also tried the ether extraction method but did not obtain any good results. Williams obtained about six per cent of lactic acid by the ether extraction method.

Higgins found it very difficult to determine the acidity of beef extract. When he titrated it directly with standard alkali he obtained the acidity due to acid phosphates and also to lactic acid. When he titrated it directly he found it hard to see the end last point as the color interferes and when he removed the color with animal charcoal some of the acid is neutralized.

If all the phosphates could be removed by means of barium carbonate paste this would be a good method but all the phosphate cannot be removed.

And finally by the ether extraction method we cannot tell whether we can get all the lactic acid out of the extract or not.

The results obtained by the different methods do not agree as can be seen from the figures just given.

So we have come to the conclusion that there is no good method known at present for the determination of the acidity of beef extract.

OBJECT OF PRESENT STUDY.

The object of this study has been to determine if possible what the acids of beef extract are and to devise some method for their quantitative determination.

EXPERIMENTAL PART.

I

Determination of volatile acids.

The determination of volatile acids includes the determination of both the free and the combined volatile acids. The method which I used was as follows:-

One hundred and one and six tenths grams of Armour's Beef Extract was weighed out transferred with about 450 cc. of pure distilled water to a two litre Schott and Genossen flask which was fitted up with a three hole rubber stopper carrying a small drop funnel, a glass tube extending nearly to the bottom of the flask for the introduction of the steam and a Hopkin's safety distilling bulb which in turn is connected with a long glass condenser, connected by means of a tight fitting cork with a tubulated receiver. A suction filter flask was used for this receiver and a Woulff bottle was connected to the filter flask. Fifty cubic centimeters of barium hydroxide (standard solution) was placed on the Woulff bottle, and a calcium chloride tube filled with potassium hydroxide was fitted in the other opening of the Woulff bottle with a tight fitting cork. The two litre flask was connected with another two litre Schott and Genossen flask for generating steam. This latter flask was provided with a long glass safety tube. It was distilled with steam and about 500 cc. collected. The volume of the liquid in the flask containing the beef extract was not allowed to fall 450 cc. The distillate was made up to exactly 500 cc. and portions of 25 cc. each titrated. Twenty-five cubic centimeters

of this filtrate was found to be equal to .02 cc. standard barium hydroxide. So I concluded that there were no free volatile acids present.

Next the combined volatile acids were determined by adding gradually through the drop funnel, 100 cc. of normal sulphuric acid while distilling with steam. The volume of the liquid in the flask containing the extract was kept as near 600 cc. as was possible but the steam condensed in the flask so a part of it had to be distilled without steam. About 1000 cc. of the distilled was collected and diluted to exactly 1000 cc., mixed thoroughly, and portions of 50 cc. each titrated using phenol-phthalein as indicator. Fifty cubic centimeters was found to be equal to .42 cc. standard barium hydroxide. Or the total 1000 cc. is equal to 8.40 cc. of the standard barium hydroxide.

The distillation was continued and 525 cc. more collected. This was titrated as before with the standard barium hydroxide solution using phenol-phthalein as indicator and it was found that 50 cc. took .40 cc. of the standard barium hydroxide or the total 525 cc. took 4.2 cc. standard barium hydroxide.

As acid was still passing over 100 cc. more of the normal sulphuric acid was added to the beef extract and 500 cc. more distilled over. The sulphuric acid was added to try and liberate the rest of the combined volatile acids. There seemed to be an excess of sulphuric acid present for the extract was acid. Twenty-five cubic centimeters of this last filtrate took .35 cc. of standard barium hydroxide or the 500 cc. filtrate was equal to 7.00 cc. of the standard barium hydroxide.

One hundred cubic centimeters more of the normal sulphuric

acid was then added to this beef extract and the mixture distilled again. Another Woulff bottle was fitted up with 50 cc. of barium hydroxide solution in it as before. About 500 cc. was distilled over and diluted up to exactly 500 cc. Fifty cubic centimeters of this was found to be equal to .55 cc. of the standard barium hydroxide. Five hundred cubic centimeters of this was therefore equal to 5.5 cc. standard barium hydroxide.

Without further addition to sulphuric acid 500 cc. more was distilled and found to be equal to 5.0 cc. standard barium hydroxide.

Acid was still coming over so without the addition of more sulphuric acid 500 cc. more was distilled and titrated with barium hydroxide, 500 cc. took 3.6 cc. of the standard barium hydroxide solution. The total amount of standard barium hydroxide required by these distillates is as follows:-

First distillate	required	8.40 cc. S. $Ba(OH)_2$.
Second	"	"	4.20 cc. S. $Ba(OH)_2$.
Third	"	"	7.00 cc. S. $Ba(OH)_2$.
Fourth	"	"	5.50 cc. S. $Ba(OH)_2$.
Fifth	"	"	5.00 cc. S. $Ba(OH)_2$.
Sixth	"	"	3.60 cc. S. $Ba(OH)_2$.
	Total		33.70 cc. S. $Ba(OH)_2$.

The solution of barium hydroxide was next standardized. The barium hydroxide was titrated against a standard solution of hydrochloric acid, using phenol-phthalein as an indicator, and 25 cc. of the barium hydroxide solution was found to be equal to 23.07 cc. of the standard hydrochloric acid.

1 cc. of the standard HCl contained .012407 gr. HCl.

From this the value of the barium hydroxide was calculated in terms of acetic acid and it was found that one cubic centimeter of my barium hydroxide solution was equal to .04277 grams of acetic acid. 73.70 cc. of the barium hydroxide was used.

73.70 x .04277 ÷ 101.6 x 100 = 1.40 % acetic acid.

From the results of this determination, I came to the conclusion that there was no free volatile acids present in beef extract.

But I found that there was 1.40 per cent of combined volatile acids present, calculated to acetic acid. This will account for a small part of the undetermined constituents.

This per cent of volatile acids which I obtained might be accounted for by the fact that when lactic acid is heated to 130° Centigrade with dilute sulphuric acid, it decomposes into aldehyde and formic acid. Formic acid is a volatile acid and so would pass over and this would account for the presence of the volatile acid.

The distillates were evaporated down after titration and the barium salt of the volatile acid obtained. It was tested for formic acid and when treated with alcohol and concentrated sulphuric acid, ethyl formate was given off which was identified by its odor. When it was treated with silver nitrate and heated, black silver oxide separated. This was filtered, washed free from the excess of silver nitrate, dissolved in nitric acid, and when hydrochloric acid was added a large precipitate of silver chloride was formed. These were characteristic tests for formic acid and seem to indicate that a part of the acid, at least, which was

obtained as a volatile acid is due to the formation of formic acid in the method described.

A pre cipitate was formed in the Woulff bottle which might have been due to the decomposition of some of the formic acid into carbon dioxide and hydrogen.

This decomposition it is stated will take place at 160° Centigrade but might possible take place at a much lower temperature. This might also apply to the decomposition of lactic acid into aldehyde and formic acid which is supposed to take place at 170° Centigrade. The temperature in my distillation did not get higher than 105° Centigrade I am positive, as there was always a large amount of water present.

DETERMINATION OF ORGANIC ACIDS BY USE OF BARIUM CARBONATE PASTE.

Next the determination of the free organic acids of beef extract was tried by digesting a sample of the beef extract with barium carbonate paste, filtering, washing the barium carbonate paste very thoroughly and then determining the barium gravimetrically in the filtrate and washings. From this the per cent of acid could be calculated figuring the free acidity as due to lactic acid. The theory on which this method is based is that the barium carbonate paste will form barium phosphate with the phosphoric acid, and acid phosphates, and as the barium phosphate is very insoluble (1 part in 10000 parts of water) it will be precipitated and so may be filtered out with the excess of barium carbonate paste.

To see if this method could be used it was tried with known amounts of primary potassium phosphate and with secondary potassium phosphate. The method that was used is as follows:- First, some barium carbonate was prepared as pure as possible. It was made into a thin paste and a blank ran upon it in the following manner:- Twenty-five cubic centimeters of the barium carbonate paste was drawn off quickly with a pipette and 100 cc. of pure distilled water added and the mixture digested upon the water bath for two hours, being careful to keep the volume up to about 100 cc. After it had digested for two hours it was filtered and the residue washed a great many times with hot distilled water. After cooling, the filtrate was diluted to a definite volume, (200 cc.) and the barium determined in this by the usual gravimetric method. There was only a very slight trace of barium

sulphate but I finished the determination and the results will be found in table No. VIII.

One hundred cubic centimeters of the filtrate from the barium carbonate digestion test was tested to see whether it was acid or alkaline. It was found to be alkaline. It was then titrated with standard acid using methyl orange as an indicator and it took .14 cc. to neutralize it or .28 cc. for the whole filtrate.

Next I tried to determine the influence of barium carbonate paste upon a solution of primary potassium phosphate. KH_2PO_4. The method was as follows:- Three or four grams of primary potassium phosphate C.P. was pulverized and dried, cooled in a dessicator, about one gram weighed off, transferred to a casserole and dissolved in fifty cubic centimeters of water. Then Thus 25 cc. of the pure barium carbonate paste was added and the whole digested upon the water bath for two hours. The solution was then filtered, the residue washed thoroughly with hot water a great many times, the filtrate and washings were diluted to a definite volume (250 cc.). The total phospheric acid was then determined in a 100 cc. portion of this filtrate by the ammonium molybdate method. I took 50 cc. of the above filtrate from this digestion and started to determine the barium in it by the usual sulphuric acid method. However, I did not obtain the slightest trace of a precipitate which shows that there was not the least trace of barium present in the filtrate. All these determinations were made in duplicate each one of the duplicates upon duplicate samples, thus giving two independent determinations.

The alkalinity was determined in a 50 cc. portion of the filtrate from the digestion of the KH_2PO_4 with the barium carbonate

paste. Two duplicate titrations were made with the filtrate from each of the samples.

50 cc. of A = .32 cc. HCl (standard)

50 cc. of A = .32 cc. HCl (standard)

50 cc. of B = .35 cc. HCl (standard)

50 cc. of B = .34 cc. HCl (standard)

Following are the results obtained:-

A	Sample	B
1.1113	Weight of sample taken	1.0989
.7762	Weight of PO_4 in sample	.7676

Sample.	Amount $LaCO_3$ paste taken.	Time digested.	Size of filtrate.	Alkalinity 50 cc.	Alkalinity whole sample.	Weight of barium in filtrate.	Weight of PO_4 in 100 cc. KH_2PO_4 filtrate.	Wt. of PO_4 in whole KH_2PO_4 filtrate.
A	25 cc.	3 hrs.	250 cc.	.32 cc.	1.60 cc.	Not a trace.	.1079	.26975
B	25 cc.	3 hrs.	250 cc.	.35 cc.	1.75 cc.		.0987	.24675

ACTION OF BARIUM CARBONATE PASTE UPON SECONDARY POTASSIUM PHOSPHATE. (K_2HPO_4)

As I was unable to obtain C.P. K_2HPO_4 I prepared it in the following manner. One gram of KH_2PO_4 was weighed out and then the precise amount of potassium hydroxide required to change it to K_2HPO_4 added. The reaction which takes place is as follows:-

$$KH_2PO_4 + KOH = K_2HPO_4 + H_2O.$$

From this equation the quantity of normal potassium hydroxide necessary to add was figured out, and it required .67 cc. of the normal potassium hydroxide. Exactly .67 cc. of the normal potassium hydroxide was added to the one gram of KH_2PO_4, which was in a little water and was boiled for some time so as to be sure to

get the reaction to take place. Then it was diluted to 200 cc. after cooling and exactly 100 cc. measured out into each of two casseroles and 25 cc. of the barium carbonate paste added to each. The determination was then continued as in the case of the KH_2PO_4 and the barium and phosphoric acid determined in the filtrate. The alkalinity was also determined and it was found that the whole sample would require .3 cc. standard hydrochloric acid. The titrations were:-

25 cc. of A = .03 cc. HCl (standard)

25 cc. of A = .03 cc. HCl (standard)

25 cc. of B = .03 cc. HCl (standard)

25 cc. of B = .03 cc. HCl (standard)

The results which I obtained were as follows:-

A	Sample	B
.6397	Weights of sample taken	.6397
.3492	Weight of PO_4 in sample	.3492

Results.

Sample.	Amount $BaCO_3$ paste used.	Weight sample K_2HPO_4 used.	Alkalinity 25 cc.	Alkalinity whole sample. (250)	Size of filtrate.	Weight Ba. in 50 cc. filtrate.	Weight Ba. in whole filtrate. (250)	Weight PO_4 in 100 cc. filtrate.	Weight PO_4 in whole filtrate.
A	25 cc.	.6397	.03 cc.	.30 cc.	250 cc.	.00066	.0033	.0485	.12125
B	25 cc.	.6397	.03 cc.	.30 cc.	250 cc.	.00066	.0033	.0481	.12025

DIGESTION TEST WITH ARMOUR'S BEEF EXTRACT.

2.6129 grams of Armour's Beef Extract was weighed out. This was suspended in 100 cc. of distilled water in a casserole.

50 cc of barium carbonate was then added and the mixture digested for two hours upon the water bath, filtered, and washed thoroughly with boiling water. The filtrate was then diluted to a definite volume (250 cc.). The barium, phosphoric acid, and alkalinity were then determined in the filtrate as in the proceeding determination. The results which were obtained will be found in the following table.

Weight of beef extract taken.	cc. $BaCO_3$ used.	Time digested.	Alkalinity 5.0 cc. filtrate.	Alkalinity whole filtrate. 250 cc.	Weight Ba. in 75 cc. filtrate.	Weight PO_4 in 75 cc. filtrate.	Weight Ba. in whole filtrate. (250 cc.)	Weight PO_4 in whole filtrate. (250 cc.)
2.6129	50 cc.	2 hrs.	.18	.9	.0068	.0132	.0230	.0440
			.18	.9	.0070		.0233	

ADDITIONAL WORK WITH PRIMARY POTASSIUM PHOSPHATE.(KH_2PO_4)

7.4156 grams of the dry C.P. KH_2PO_4 was taken and made up to exactly 500 cc. A solution was obtained from which a portion could be taken and the amount of KH_2PO_4 contained in it would be known.

Two sets of determination were made which were just the same except that 50 cc. instead of 25 cc. of the barium carbonate paste was used in the second determination. The method which was used in this set of determinations was as fo lows:-

I. 25 cc. of the KH_2PO_4 solution was taken in duplicate. This amount of the KH_2PO_4 solution contained .3416 grams of KH_2PO_4. 25 cc. of pure distilled water and 25 cc. of pure barium carbonate. paste was added and the mixture digested upon the water bath for

exactly three hours keeping the volume of the liquid about constant at 75 cc. by the addition of distilled water. It was then allowed to stand in the cold for twenty-one hours. It was then filtered and washed very thoroughly with cold water, the filtrate and washings diluted to a definite volume (250 cc.). The total phosphoric acid was then determined in 50 cc. portion of the filtrate, in duplicate, by the ammonium molybdate method. The acidity or alkalinity was determined in 50 cc portions in duplicate. In the same portion the barium was determined by acidifying with hydrochloric acid and precipitating with dilute sulphuric acid. The results will be found in table No. VIII.

II. I repeated the above method in duplicate using 50 cc. of the pure barium carbonate paste instead of 25 cc. as in the above. The barium, phosphoric acid, and alkalinity were determined in the filtrate as before. The results of this determination will be found in table No. VIII.

III. Next I ran a blank upon the barium carbonate paste following the method given in I, but of course did not take any of the KH_2PO_4 solution. The barium and alkalinity were determined in the filtrate but of course there was no phosphoric acid. The results of this determination will be found in table No. VIII.

Table No. VIII.

Action of phosphates on barium carbonate paste, and as to whether barium carbonate paste could be used to determine the organic acids of beef extract which form soluble barium salts.

BLANK ON BARIUM CARBONATE PASTE.

25 cc. of barium carbonate paste digested two hours upon the water bath.

Sample.	Amount of $BaCO_3$ paste taken.	Time digested.	Size of filtrate.	Alkalinity.	Weight Ba. in 100 cc. filtrate.	Weight Ba. in whole filtrate.
A	25 cc.	2 hrs.	200 cc.	.28 cc.	.01259	.02513
B	25 cc.	2 hrs.	200 cc.	.28 cc.	.01247	.02494

Primary potassium phosphate. (KH_2PO_4)

25 cc. barium carbonate paste digested with sample for three hours.

Sample.	Weight sample. KH_2PO_4	Alkalinity. 50 cc.	Alkalinity whole sample.	Ba. in filtrate.	Weight PO_4 in 1000 cc. KH_2PO_4 filtrate.	Weight PO_4 in whole KH_2PO_4 filtrate.
A	1.1113	.32 cc.	1.60 cc.	Not a trace.	.1079	.26975
B	1.0989	.35 cc.	1.75 cc.		.0987	.24675

Table No. VIII. (continued)

SECONDARY POTASSIUM PHOSPHATE (K_2HPO_4)

25 cc. barium carbonate paste digested with sample for three hours.

Det.	Weight sample K_2HPO_4.	Alkalinity whole sample.	Weight Ba. in 50 cc.	Weight Ba. in whole filtrate.	Weight PO_4 in 100 cc. K_2HPO_4 filtrate.	Weight PO_4 in whole filtrate. (250)
A	.6397	.30 cc.	.00066	.0033	.0485	.12125
B	.6397	.30 cc.	.00066	.0033	.0481	.12025

DIGESTION TEST WITH ARMOUR'S BEEF EXTRACT.

2.6129 grams extract taken and digested with 50 cc. of barium carbonate paste for two hours upon the water bath.

Det.	Weight of sample beef extract taken.	Alkalinity whole filtrate.	Weight Ba. in 75 cc. filtrate.	Weight Ba. in whole filtrate.	Weight PO_4 in 75 cc. filtrate.	Weight PO_4 in whole filtrate.
A	2.6129	.9 cc.	.0068	.0330	.0172	.0440
B		.9 cc.	.0070	.0333		

ADDITIONAL WORK WITH PRIMARY POTASSIUM PHOSPHATE.(KH_2PO_4)

Determination No. I.

25 cc. barium carbonate paste digested with sample for exactly three hours and allowed to stand in the cold twenty-one hours.

Det. No.	Weight of sample of KH_2PO_4 taken.	cc. $BaCO_3$ paste used.	Weight PO_4 in 50 cc. filtrate.	Weight PO_4 total filtrate. (250 cc.)	Weight Ba. in 50 cc. filtrate.	Weight Ba in total filtrate. (250 cc.)	Alkalinity.
A	.74156	25 cc.	.01299	.06495	.001129	.005645	.72
B	.74156	25 cc.	.01282	.06410	.001240	.006230	.71

Determination No. II.

50 cc. barium carbonate digested with sample for exactly three hours and allowed to stand in the cold for twenty- one hours.

Det.	Weight of sample of KH_2PO_4 taken.	cc. $BaCO_3$ paste used.	Weight PO_4 in ~~total~~ 50 cc. filtrate. ~~250 cc.~~	Weight PO_4 in ~~50 cc.~~ total 250 cc. filtrate.	Weight Ba. in 50 cc. filtrate.	Weight Ba. in total filtrate. (250 cc)	Alkalinity.
A	.74156	50 cc.	.01115	.05575	.00124	.0062	.80
B	.74156	50 cc.	.01107	.05535	.00059	.002952	.86

Determination No. III.

BLANK ON BARIUM CARBONATE PASTE.

25 cc. barium carbonate paste taken, digested for exactly three hours on water bath and allowed to stand in cold for twenty-one hours.

No. of determination.	cc. $BaCO_3$ paste taken.	Ba. in 50 cc. filtrate.	Ba. in whole filtrate.	Alkalinity.
A	25 cc.	.00224	.0172	.70
B	25 cc.	.00207	.01035	.74

From these results it can be seen that when a solution of KH_2PO_4 is digested with barium carbonate paste, and the barium and phosphoric acid determined in the filtrate and washings, a small quantity of barium was found in the filtrate. The phosphosphate and alkalinity might be accounted for in that potassium carbonate and potassium phosphate might have been formed and these being easily soluble would be found in the filtrate. When barium carbonate acts upon KH_2PO_4 this reaction might have taken place.

$$7KH_2PO_4 + 5BaCO_3 = 5BaHPO_4 + 2K_3PO_4 + KHCO_3 + 4CO_2 + 4H_2O.$$

Now the $BaHPO_4$ is soluble in 10000 parts of water and so would all be removed by filtration, as insoluble $BaHPO_4$. But the phosphoric acid was not all removed by the action of the barium carbonate paste and the only way in which I could account for this is that neutral potassium phosphate was formed, by above equation, which is soluble but probably would not react with $BaCO_3$ paste, and so would remain in solution.

According to these results, this method could not be used, to determine the free and organic acids in extracts containing primary and secondary phosphates, as it failed to remove all the phosphoric acid, as we thought it would do. It might be that if the conditions were varied in some way that the method could be made to work.

TOTAL ACIDITY OF BEEF EXTRACT.

Determined by direct titration of a solution of the extract with standard alkali.

I attempted to determine the total acidity of beef extract by titration with standard alkali. One method which I used was as follows:-

Thirty grams of Armour's beef extract was dissolved in 200 cc. of distilled water, the solution was diluted to 250 cc. and it was then mixed thoroughly. This solution was labelled "A". Twenty five cubic centimeters of solution "A" was taken, in duplicate and barium hydroxide solution was added in slight excess. This removed the phosphates by precipitating them as barium phosphate. It was allowed to stand for about twenty one hours, and then filtered and the precipitate washed thoroughly with cold water. It was then treated again with a little barium hydroxide solution to be sure that all the phosphates had been removed from the filtrate. The filtrate and washings were then diluted to a definite volume (250 cc.). This solution was labelled "B".

Two hundred cubic centimeters of solution "B" was boiled. Then a slight excess of dilute sulphuric acid was added. It was then filtered and washed thoroughly with boiling water. This removed the excess of barium hydroxide. The filtrate was then evaporated, in a proper sized porcelain dish, upon the water bath. It was then diluted to definite volume (250 cc.) and this solution was labelled "C".

Ten cubic centimeter portions of solution "C" were titrated with standard potassium hydroxide, using phenol-phthalein

as an indicator. From these results the amount of standard potassium hydroxide required to neutralize 200 cc. of solution "C" was figured out. It was found that 10 cc. of solution "C" required 7.03 cc. normal potassium hydroxide. 200 cc. of solution "C" would therefore require 20 x 7.03 = 140.6 cc. of normal potassium hydroxide to neutralize it. This amount of standard potassium hydroxide and 25 cc. in addition was added to 200 cc. of solution "C". This was then evaporated to dryness in a three and one half inch porcelain dish. It was then dried in the oven at 100° to 105° C. and ignited carefully until thoroughly charred. The charred residue was digested with hot water but the water was so black that a titration could not be made, and so this method cannot be used. The theory on which this method was based, was to convert the acids present into carbonates by the excess of alkali and then by charring to remove the organic coloring matter. Thus to extract and dilute extracts to a definite volume say 250 cc., mix the filtrate thoroughly and titrate with standard hydrochloric acid as follows:- Take 25 cc. portions, add an excess of standard hydrochloric acid, boil thoroughly, and determine excess of hydrochloric acid by titration, with standard sodium hydroxide. Owing to the colored solution this method could not be used.

Next I tried to remove the coloring matter by filtering through precipitated silica. A good sized filter was fitted in a three inch funnel. The funnel was filled two thirds full of precipitated silica, washed thoroughly with dilute hydrochloric acid (1 of dilute HCl to 10 of water) and then with hot water until the acid was all removed. Then 10cc. of solution "A" was diluted

with 50 cc. of water, filtered through the washed precipitated silica but the silica failed to remove the coloring matter, and so the method would not work.

A filter was next fixed as in the case of the precipitated silica but instead of using precipitated silica, Fuller's earth was used. The hydrochloric acid could not be washed out as it filtered so slowly. So a piece of filter paper was fitted to a Büchner funnel and the Fuller's earth placed upon it. But the Fuller's earth packed so firmly that it could not be washed. Next a Hirsh funnel was used but it packed closely as in the case above and so could not be used. Then a Gooch funnel was used with a piece of filter paper over the lower openings, but again the Fuller's earth packed so firmly that nothing could be done with it. So a layer of asbestos pulp was placed in the bottom of the Gooch funnel, and then a small layer of Fuller's earth upon this but the Fuller's earth packed as before and nothing could be done with it. Then the Fuller's earth and asbestos pulp were mixed and then placed in the Gooch funnel but it packed so tightly that little could be done with it. However, the acid was finely washed out of it but the Fuller's earth failed to remove the coloring matter of the beef extract so we gave up the method of filtration to remove the coloring matter.

Next the weight of barium chloride to react with one gram of potassium sulphate was calculated, the water of crystallization being taken into account. One gram of potassium sulphate and one half the calculated quantity of barium chloride ($BaCl_2 \cdot 2H_2O$) were taken. Each dissolved separately in a small quantity of hot water.

The potassium sulphate solution added to the cold solution of 10 cc. of solution "A" in 100 cc. of water, then the barium chloride solution added, the solution stirred thoroughly and allowed to stand over night. The solution was then filtered but the precipitate of barium sulphate came through the filtre so badly that nothing could be done with it. The purpose of the method was to form a precipitate which would carry the organic matter down with it and then this could be filtered, out and the clear solution titrated, but the method could not be used as the precipitate came through the filter paper and did not remove the organic coloring matter anyway. However, the precipitate was stirred up and formed a white back-ground which when the solution was titrated with standard alkali showed the end reaction of the phenol-phthalein quite well.

10 cc. of Sol. "A" required 13.5 cc. N/10 KOH.

250 cc. of Sol. "A" required 337.5 cc. N/10 KOH.

250 cc. of Sol. "A" contains 30.7060 grams Beef extract

1 grams beef extract requires 10.9913 cc. N/10 KOH.

100 grams beef extract requires 1099.13 cc. N/10 KOH.

1 cc. N/10 $CH_3CHOHCOOH$ contains .009006 grams lactic acid.

1099.13 x .009006 = 9.8913 grams lactic acid, per 100 grams of beef extract.

Next five solution of beef extract were made up using different numbers of cubic centimeters of solution "A". The barium chloride and potassium sulphate were added as before and the barium sulphate was thrown down as a white precipitate. All the solutions were in large beakers of the same size, and were diluted to the same volume with distilled water. The volume was about 400 cc.

They were then titrated with one-tenth normal potassium hydroxide using phenol-phthalein as an indicator. The titrations were as follows:-

No. 1. - 8 cc. Sol. "A" required 15.7 cc. N/10 KOH.

No. 2. - 5 cc. Sol. "A" required 10.60 cc. N/10 KOH.

No. 3. - 5 cc. Sol. "A" required 10.30 cc. N/10 KOH.

No. 4. - 9 cc. Sol. "A" required 16.3 cc. N/10 KOH.

No. 5. - 10 cc. Sol. "A" required 16.7 cc. N/10 KOH.

The grams of lactic acid per 100 grams of beef extract were calculated from this as in the case proceeding and will be found in the following table.

No. of Det.	cc. of Sol. "A" taken.	Titration with N/10 KOH.	How titrated.	Grams of lactic acid per 100 grs. of beef extract.
1.	8	15.7	Barium sulphate precipitate.	14.3699
2.	5	10.6		15.5443
3.	5	10.3		15.1030
4.	9	16.3		13.27
5.	10	16.7		12.24
"	10	13.5		9.89

It was much easier to tell the end point when five or less than five cubic centimeters of solution "A" were used as the solution was not so colored and the results obtained agree closed. This method I believe is good if a small amount of the solution is used and the solution diluted to about 400 cc.

Next the acidity was determined by directly titrating a small amount of solution "A" which was diluted a great deal,

using phenol-phthalein as an indicator. It was rather hard to tell just when the end point was however. Ten cubic centimeters of solution "A" were diluted to 200 cc. and titrated with standard alkali. 10 cc. Sol. "A" required 19.00 cc. N/10 KOH. From this the number of grams of lactic acid per one hundred grams of beef extract was calculated, and it was found that this gave 17.92 grams of lactic acid for one hundred grams of beef extract.

Next three solutions were made up with different numbers of cubic centimeters of solution "A" in them, the amount being unknown to me until after I had titrated them. They were then diluted to about 400 cc. and titrated direct with standard alkali using phenol-phthalein as an indicator. The results of the titrations were:-

No. 1. Contained 5 cc. Sol. "A" required .85 N KOH.

No. 2. Contained 10 cc. Sol. "A" required 1.80 cc. N KOH.

No. 3. Contained 8 cc. Sol. "A" required 1.60 cc. N KOH.

No. of Det.	cc. Sol. "A" used.	Titration with N KOH.	How titrated.	Grams of lactic acid per 100 grams of beef extract.
10.	10	19 cc. N KOH		17.92
1.	5	.85	Direct	12.45
2.	10	1.80		13.19
3.	8	1.60		14.65

Thus by a comparison of this and the proceeding table it will be seen that the results obtained by direct titration with a back ground of barium sulphate give practically the same results

which vary from twelve to fifteen grams of lactic acid per one hundred grams of beef extract on one and the same sample.

Portions of solution "A" after diluting 200 cc. were titrated with standard alkali using in this case methyl orange as indicator. It could not be titrated, however as the color interferred so that the end reaction could not be told.

Next a lactic acid extraction was diluted to exactly 50 cc. and mixed thoroughly. 10 cc. portions were taken in duplicate and titrated as usual using phenol-phthalein as indicator, and the following results were obtained:-

10 cc. took 5.95 cc. N/10 KOH.

10 cc. took 5.94 cc. N/10 KOH.

Next ten cubic centimeter portions of the solution were taken and titrated as in the above case only methyl orange was used as an indicator instead of phenol-phthalein. I could not tell where the end reaction was as there was a sort of fading of the red into yellow which appears to get deeper for a time with the addition of alkali.

The results obtained by these various methods show that there is about 14 grams of acid per one hundred grams of extract when calculated to lactic acid. This is in a large measure due no doubt to acid phosphates. I tried to determine the lactic acid by means of extraction and the difference between the results I obtain will give some idea of the relative amounts of lactic acid and acid phosphate. The only methods which seemed to work were direct titration and titration with a precipitate of barium sulphate. These methods are the best and the results obtained by them agree well.

DETERMINATION OF ORGANIC ACIDS OF BEEF EXTRACT BY EXTRACTING WITH ETHER.

In the next place, I attempted to determine the organic acids of beef extract by extracting with ether and titrating the ether extracts with standard alkali solution. The method which I used was as follows:-

Five hundred grams of Armour's beef extract was transferred to a two litre Schott and Genossen flask. Five hundred cubic centimeters of water was added and the mixture shaken thoroughly. Then 1000 cc. of strong alcohol was added and the mixture shaken thoroughly. The mixture was heated upon the water bath for three hours with frequent shaking. It was then allowed to stand over night. The solution was then filtered and the residue washed four times, with 60 per cent alcohol. The filtrate and washings were then evaporated to a syrup and then this residue was digested with about 250 cc. of strong alcohol. It was warmed upon the water bath with frequent stirrings. Then it was cooled and 100 cc. of strong alcohol added and stirred thoroughly, and allowed to stand until the liquid was as clear as it would get. It was then filtered and the residue was washed carefully five times with strong alcohol. The filtrate and washings were then evaporated to a syrup. The mass became very thick and crystals of some substance formed. It looked as if there might be some residue present which would not dissolve in alcohol. To make sure that it was all soluble in alcohol, some alcohol was added to the mass and then the mass was heated upon the water bath. It all went into solution showing that it was soluble in alcohol. It was then evaporated to a syrup and

and transferred to a large separatory funnel with as little water as possible. Then 500 cc. of ether was added and the mixture shaken well for about fifteen minutes. It was then allowed to stand over night so that the ether would separate as much as possible from the residue. It did not separate very well, but about 200 cc. of the ether was poured off into a 250 cc. Erlenmeyer flask. Then the ether was distilled off and the residue in this flask dissolved in 50 cc. of water. After heating it to boiling, it was titrated with one tenth normal potassium hydroxide using phenol phthalein as indicator. It required 23.95 cc. of the one tenth normal potassium hydroxide to neutralize the acid present. Five hundred cubic centimeters more of ether was added to the residue from this ether extraction and the mixture was shaken up as before. It was allowed to stand over night and the ether was poured off into a flask. The ether was then distilled off and the residue in the flask was titrated as in the proceeding case. This was done sixteen times and the results of the titrations of the extractions are given below.

1st	extraction required	23.95 cc.	N/10 KOH.
2nd	" "	32.80 cc.	" "
3rd	" "	38.58 cc.	" "
4th	" "	34.80 cc.	" "
5th	" "	28.00 cc.	" "
6th	" "	29.75 cc.	" "
7th	" "	12.20 cc.	" "
8th	" "	7.50 cc.	" "
9th	" "	6.05 cc.	" "

10th extraction required 9.50 cc. N/10 KOH.

11th " " 10.10 cc. " "

12th " " 10.40 cc. " "

13th " " 6.40 cc. " "

14th " " 6.20 cc. " "

15th " " 8.20 cc. " "

16th " " 7.50 cc. " "

Total No. of cc. N/10 KOH. 271.93.

This was calculated to grams of lactic acid per one hundred grams of beef extract. This gave me the amount of free acid present in beef extract.

1 cc. N/10 $CH_3CHOHCOOH$ contains .009006 grams. .009006 x 271.93 = 2.449 grams of lactic acid in 500 grams of beef extract. 2.449 ÷ 5 = .4898 grams lactic acid per 100 grams beef extract or .4898% free lactic acid.

Next 10 cc. of the liquid from the first extraction was tested to see if there were any phosphates present but no phosphates were found. It was tested with molybdate solution and nitric acid.

Ten cubic cubic centimeters of the liquid was tested to see if the acids were organic acids. I found that they were for it charred and gave off the odor of burning material when heated. It left a very minute quantity of white ash.

Ten cibic centimeters of the liquid was tested to see if there was any hydrochloric acid present but none was found. Tested with silver nitrate and nitric acid.

Ten cubic centimeters was tested to see if there was any sulphuric acid present but none was found, with barium chloride

and hydrochloric acid.

The extract was tested further for tartaric acid but none was found. It was tested for the odor of burnt sugar when ignited but gave no such odor. Silver nitrate did not give a precipitate.

No acetic acid was present; for when the extract was digested with alcohol and sulphuric acid it did not give the odor of acetic ether and it did not give a red solution with ferric salts.

No boric acid was present; and when the extract was mixed with alcohol and the alcohol set on fire it gave no green flame.

No citric acid was found. Silver nitrate did not give any precipitate with the extract but if citric acid had been present a white precipitate of silver citrate would have been formed.

No oxalic acid was present. Carbon dioxide was not given off when the solution was heated as oxalates would do.

I found that the acid was lactic acid. This was proven by two methods. Uffelmann's test, which is a very delicate test was one which was used. The test was made as follows:- The reagent was prepared by mixing 10 cc. of a 4 per cent solution of carbolic acid with 20 cc. of water, and adding a drop of a solution of ferric chloride C.P. This forms a clear liquid of an amethyst color which is turned yellow by a solution of lactic acid, containing only 1 part in 10,000.

Lactic acid was also found to be present by means of the following method, which is also a quantitative method for determining lactic acid. The solution to be tested for lactic acid was evaporated to dryness upon the water bath then it was taken up with alcohol. To this a solution of lead acetate and alcoholic ammonia

was added, a white precipitate was produced indicating the presence of lactic acid. The alcoholic ammonia is made by passing ammonia vapors into absolute alcohol. There must not be any water present or basic lead acetate will be thrown down as a white precipitate. Lactic acid was found to be the acid in the extract by both of these methods.

The extraction of lactic acid by means of ether.

The object of my work with lactic acid was to see if all the lactic acid would be extracted by ether. To find this out, 2 grams of C.P. lactic acid was dissolved in 100 cc. of distilled water and mixed very intimately. Then 10 cc. portions of this were taken and diluted with 25 cc. of distilled water and after heating to boiling were titrated with standard alkali, in this case one tenth normal potassium hydroxide was used. Titrations were run upon 10 cc. portions until three were obtained which agreed very closely. These were then evaporated down to 2 or 3 cc. and transferred, with a few drops of water, to a small separatory funnel. The solution was then acidified with sulphuric acid. It was then extracted a number of times with ether using 25 cc. of ether for each extraction. When ten extractions had been made the total extractions were washed with a few cubic centimeters of water to take out any sulphuric acid that might be present. The ether was di tilled off and water was then added to the residue. It was then heated to boiling and titrated with one tenth normal potassium hydroxide. This was done a great many times and the results will be found below.

Results.

1st 10 cc. lactic acid solution required 16.05 cc. N/10 KOH.

2nd 10 cc. lactic acid solution required 16.06 cc. N/10 KOH.
3rd 10 cc. " " " " 16.10 cc. " "
Total 48.19 cc. " "

Extractions.

1st 8 extractions required 19.2 cc. N/10 KOH.
Next 10 " " 15.88 " " "
" 10 " " 2.42 " " "
" 10 " " .65 " " "
" 10 " " 4.06 " " "
Total 42.21 " " "

Lactic acid taken required 48.19 cc. N/10 KOH.
Extractives required 42.21 " " "
Still unextracted 5.98 " " "

2.0274 grams of lactic acid was the amount weighed out.

The result for the last ten extractions was high because there was a little sulphuric acid in the residue which was not removed before titrating.

Next I again weighed out 2.0169 grams of C.P. lactic acid and proceeded as in the above determination. The results which I obtained were.

Results.

1st 10 cc. lactic acid solution required 16.10 cc. N/10 KOH.
2nd 10 cc. " " " " 16.12 " " "
3rd 10 cc. " " " " 16.10 " " "
Total 48.32 " " "

Extractions.

1st extractions required 15.95 cc. N/10 KOH.

2nd extractions required	3.50	cc.	N/10	KOH.	
3rd " "	17.30	"	"	"	
4th " "	4.60	"	"	"	
5th " "	2.50	"	"	"	
6th " "	.93	"	"	"	
7th " "	.85	"	"	"	
8th " "	.50	"	"	"	
9th " "	.35	"	"	"	
Total	42.53	"	"	"	
Lactic acid taken required	48.32	cc.	N/10	KOH.	
Extractions required	42.53	"	"	"	
Still unextracted	4.79	"	"	"	

70 extractions were made in this determination.

These results show that ninety per cent of the lactic acid can be extracted by ether. But that ten extractions will only remove about thirty percent. In the method which is used by most of the investigators for the determination of lactic acid, the residue in which the lactic acid is to be determined is extracted from 5 to 8 times with ether. However from my determinations these results appear to be low and would also indicate that they only obtained thirty per cent of the pactic acid present. As at least ninety per cent of the lactic acid could be removed by extraction with ether, the residue should be extracted a great many times, or until the titration shows that all the acid has been extracted by the ether.

ESTIMATION OF COMBINED ORGANIC ACIDS IN BEEF EXTRACT.

Next the residue of beef extract left from the determination of the free acids of beef extract by extraction with ether was acidified with sulphuric acid. This was done by adding 25 cc. of dilute sulphuric acid to the residue. Then 500 cc. of ether was added and the mixture was shaken well for about fifteen minutes. The mixture was allowed to stand over night and the ether was poured off next day into a proper sized flask. The ether was washed twice with about 5 cc. of distilled water to remove any sulphuric acid which the ether might have taken with it. The ether was then distilled off and the water was added to the flask. The water was heated to boiling and the residue was then titrated with one tenth normal potassium hydroxide using phenol-phthalein as an indicator. This was repeated a great many times and the results will be found below.

Results.

1st	extraction	required	19.40	cc. N/10	KOH.
2nd	"	"	112.70	" "	"
3rd	"	"	91.20	" "	"
4th	"	"	87.50	" "	"
5th	"	"	64.80	" "	"
6th	"	"	88.80	" "	"
7th	"	"	90.60	" "	"
8th	"	"	56.30	" "	"
9th	"	"	34.90	" "	"
10th	"	"	79.60	" "	"
11th	"	"	97.90	" "	"

12th extraction required	19.40	cc.	N/10	KOH.
13th	"	"	43.00	"	"	"
14th	"	"	59.15	"	"	"
15th	"	"	67.00	"	"	"
16th	"	"	51.20	"	"	"
17th	"	"	43.10	"	"	"
Total	1083.65	"	"	"

After I had extracted nine times I added 25 cc. more of dilute sulphuric acid so as to be sure that the residue was acid. I added more ether from time to time as the ether was lost by distillation.

The 1083.65 cc. of N/10 KOH which was taken by the total extracts was calculated to grams of lactic acid per one hundred grams of beef extract. This gave me the amount of combined lactic or organic acids present in beef extract - 1 cc. N/10 $CH_3CHOHCOOH$ contains .009006 gram. .009006 x 1083.65 = 9.7594 grs. of lactic acids in 500 grs. of beef extract. 9.7594 ÷ 5 = 1.95 grs. of lactic acid per 100 grams of beef extract, or 1.95% combined lactic acid.

By my previous work I obtained .48% free lactic acid which would make a total of 1.95% combined lactic acid.

.48% free lactic acid.

2.43% lactic acid.

This determination of combined organic acids is still in an uncompleted state as acid is still being extracted by the ether but owing to lack of time I was unable to complete the determination.

In concluding it may be well to make a brief summary of the results of this work.

1st I found that there was 1.4% combined volatile acids calculated to acetic acid, in Armour's beef extract but that there was no free volatile acids present.

2nd That the Barium Carbonate paste method for the determination of free organic acids would not work as it did not remove all the phosphates.

3rd That the methods in use I use for the determination of lactic acid by means of ether extraction are not good as only about 30% of the acid is extracted by the ether in six extractions. However I was able by means of a great number of extractions to obtain 90% of the lactic acid in a sample of C.P. lactic acid.

4th The total amount of lactic acid obtained by extraction with ether was 2.45%.

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